STUDIES ON COCCOBACILLUS ACRIDIORUM D'HERELLE, AND ON CERTAIN INTESTINAL ORGANISMS OF LOCUSTS.

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PART I. EXPERIMENTS ON THE CONTROL OF LOCUSTS BY THE USE OF COCCOBACILLUS ACRIDIORUM D'H.

A. Historical Resume.

Coccobacillus acridiorum, the causal organism of an epizootic disease of locusts, was isolated in the State of Yucatan, Mexico, in 1910, by Dr. F. d'Herelle from the South American migratory locust *Schistocerca americana* Drury. He had observed that during the previous year the swarms migrating northward into Mexico from the confines of Guatemala showed evidence of the presence of an epizootic. The mortality in the swarms increased each year until 1912, when the disease had destroyed the locusts to such an extent that no swarms migrated into Mexico. D'Herelle was able to produce disease and death by inoculating healthy locusts with a culture of the organism which he isolated from the diseased locusts. The results of his experiments led him to believe that the use of this organism would have successful results in the control of locusts.

In 1911–12 he was given an opportunity to test the effectiveness of his cultures against *Schistocerca paranensis* Burm. in the province of Santa Fe, Argentina, where his attempts met with a decided success.

Results obtained by Sergent and Lheritier in Algeria during 1913 were not conclusive. They found that *Dociostaurus maroccanus* Thunberg was susceptible to the disease, but the epizootic did not spread with sufficient rapidity to cause appreciable diminution in the size of the swarms. They attributed their failure to three contingencies, either the infection did not spread through the greater portion of the migrating swarm, or many of the locusts possessed a natural immunity, or else they easily acquired an active immunity against the organism.

Lounsbury in 1913 conducted experiments in South Africa to determine whether *C. acridiorum* could be effectively used in combating the non-migratory *Zonocerus elegans*. His exper-

iments were unsuccessful as the disease did not spread in the field, and he came to the conclusion that under South African conditions the biological method of d'Herelle can be used only as a supplementary measure and cannot supersede the use of poison baits in the control of locusts.

Oedaleus nigrofasciatus De Geer and *Locusta migratoroides* R. and F., two injurious locusts in the Philippines, were experimented on by Barber and Jones in 1913. An absolute failure in the field experiments was reported.

The Entomological Branch of Canada attempted without success to introduce the disease in parts of Quebec during the seasons of 1913 and 1914. Owing to the fact that the culture had to be sent a considerable distance from the laboratory in which it was prepared, which would probably affect the virulence of the organism, no definite conclusions were reached.

In 1914–15 Beguet, Musso and Sergent conducted a campaign in Algeria against an invasion of *Schistocerca peregrina* Ol. using both the biological and the mechanical methods of control. The combination of the two methods proved very successful. The biological method could not be used to protect fields that were directly menaced as the disease spread slowly. In the Sebdou region two indigenous coccabacilli were found which immunized the locusts against d'Herelle's organism. Similar organisms were reported from Algiers.

During 1915 a locust invasion of Tunisia threatened disaster over about 36,000 square miles of territory. D'Herelle succeeded in completely controlling the outbreak by means of a combination of the biological and mechanical methods.

In Morocco during 1915 Velu and Bouin conducted extensive experiments on the control of *S. peregrina*. They concluded that "d'Herelle's method gives encouraging results. Starting with a sufficiently virulent culture of the coccobacillus it is possible to create, either by spraying with bouillon or by contamination from diseased nymphs, an epizootic which is very contagious and sometimes extremely deadly, but the progress of which is by no means overwhelming." They advise its judicious combination with other methods.

The experiments described below were conducted at the request of Dr. C. Gordon Hewitt, Dominion Entomologist, during the summer of 1916. The original culture used was obtained by Dr. Hewitt from the Pasteur Institute at Paris.

B. Symptoms of the Disease.

The time elapsing between infection and the manifestation of the symptoms characteristic of the disease depends on the virulence of the organism, and may vary from a few hours to several days. Diseased locusts become sluggish and more or less paralyzed, losing to some extent the power of leaping. The excrement is black and fluid, and when the insect is dissected it is found that the contents of the digestive tract are black and more or less slimy. After death putrefaction proceeds rapidly and the integument becomes blackened.

Bacteriological or microscopical examination reveals the presence of the coccobacillus in the intestinal tract, the blood and faeces in practically pure culture.

C. Increasing the Virulence of the Organism.

It was the experience of d'Herelle and subsequent workers that the coccobacillus when grown in artificial culture media becomes very much weakened, but that the virulence could be progressively increased by passing the organism through a succession of locusts.

In order then to obtain a culture sufficiently strong for our experiments it was necessary thus to increase the virulence. The first lot of locusts was inoculated with a suspension of the original culture. On analyzing the contents of the intestines of locusts killed by this injection we obtained a pure culture of the coccobacillus. We decided then to use a suspension of the intestinal contents of the dead locusts in our further injections. Parallel with this we ran what we termed "a pure culture series," that is, a series in which the intestinal contents of the dead locusts were plated out on 1% beef peptone agar, incubated at 30° C. for eighteen hours, and then from the plates a typical colony selected and this pure culture used for inoculating the next lot. By the first method we obtained a virulent culture much sooner than by the second.

Our method of procedure was as follows: The dead locust was placed for a few minutes in alcohol. Upon removal from the alcohol its body was split along the back with a sterile pair of scissors and a portion of the digestive canal severed. The cut portion was removed with sterile forceps, dropped in a test tube containing 10 cc. of sterilized water and triturated. The suspension thus obtained was used in inocluating the healthy locusts.

The locust to be inoculated was held between the thumb and forefinger of the left hand and a drop of the suspension was injected between the first and second abdominal sternites by means of a very fine hypodermic needle.

The first lot inoculated were all dead in five days, owing doubtless to the fact that a rather strong suspension of the original pure culture was used. The second, third and fourth lots did not all die, some remaining alive for upwards of twenty-three days. After the fourth inoculation no injected locusts survived.

Some of the locusts of the second, third and fourth lots which were apparently healthy after twenty-three days were killed and the intestinal contents examined, and we found that the coccobacillus was present.

The remainder of the survivors were injected with a virulent culture and all died within a few hours.

The following table shows the increase of the virulence of the organism. The first lot in the series was inoculated with Dr. d'Herelle's culture, the others were each inoculated with a suspension of the intestinal contents of the preceding lot.

TABLE I.

SHOWING THE INCREASE IN THE VIRULENCE OF *C. acridiorum*, TEMPERATURE ABOUT 85°F

Lot	No. inoculated	Time	No. dead	Time	No. dead	Time	No. dead	Time	No. dead	Time	No. dead	Time	No. dead	Last one dead
1	12	1 day	4	2 days	6	3 days	8	4 days	9	5 days	12	.....	...	5 days
2	12	8 hrs.	2	16 hrs.	5	27 hrs.	6	2 days	7	4 days	8	5 days	9	*
3	14	20 hrs.	6	27 hrs.	7	3 days	9	3⅓ da.	10	.....	...	.....	...	*
4	15	22 hrs.	3	46 hrs.	4	71 hrs.	5	6 days	6	.....	...	.....	...	*
5	15	21 hrs.	9	23 hrs.	11	28 hrs.	14	34 hrs.	15	.....	...	.....	...	34 hrs.
6	20	5 hrs.	1	9 hrs.	4	21 hrs.	16	27 hrs.	20	.....	...	.....	...	27 hrs.
7	12	6 hrs.	1	8 hrs.	6	96 hrs.	9	10 hrs.	10	11 hrs.	12	.....	...	11 hrs.
8	12	15 hrs.	12	.....	...	.....	...	.....	...	.....	...	.....	...	†
9	15	5 hrs.	3	7 hrs.	5	8 hrs.	12	10 hrs.	14	11 hrs.	15	.....	...	11 hrs.
10	15	3 hrs.	2	8 hrs.	10	10 hrs.	14	11 hrs.	15	.....	...	.....	...	11 hrs.
11	12	4 hrs.	1	6 hrs.	4	8 hrs.	5	10 hrs.	8	13 hrs.	10	19 hrs.	12	between 13&19hr ‡
12	14	2 hrs.	2	7 hrs.	10	9 hrs.	14	.....	...	.....	...	.....	...	9 hrs.

*Did not all die.

†No observation made until 15 hours after injection.

‡No observation made between the 10th and 19th hours after inoculation. The temperature on the day this lot was inoculated fell nearly 10°F., which accounts for the longer time required for death to occur.

After the twelfth lot there was little increase in virulence. Quite often several locusts would die within a very short while after being injected. This was probably due to a previously weakened condition of the locusts which rendered them less resistant to the septicæmic action of the coccobacillus. The intestinal contents of those which died thus early were not as virulent as those of the ones which died later. The following table brings out this point:

TABLE II.

COMPARISON BETWEEN THE VIRULENCE OF THE INTESTINAL CONTENTS OF LOCUSTS WHICH DIE VERY EARLY AND OF THOSE FROM THE SAME LOT WHICH DIE LATER.

Source of culture	No. inoculated	Time	No. dead	Time	No. dead	Time	No. dead	Remarks
Early killed locusts.....	12	3 hrs.	1	11 hrs.	2	24 hrs.	11	1 alive after 24 hours.
Later killed locusts.....	14	2 hrs.	2	7 hrs.	10	9 hrs.	14	All dead in 9 hours.

D. Insects Susceptible to the Disease.

The pathogenicity of *Coccobacillus acridiorum* was tested for all species of locusts and grasshoppers commonly occurring in large numbers in this region. These were *Melanoplus femur-rubrum, M. bivittatus, M. atlanis, Dissosteira carolina, Camnula pellucida, Stenobothrus curtipennis* and *Xiphidium sp.* All of these insects proved to be susceptible.

Gryllus pennsylvanicus, one of the common field crickets, also died as a result of injection with the coccobacillus, and several dead specimens of *Nemobius spp.* were found dead in the field, doubtless as a result of eating the infected bran mash.

Of insects other than the Orthoptera only two were tested, the yellow bear caterpillar (*Spilosoma virginica*) and the potato beetle (*Leptinotarsa decemlineata*) larvæ and adults. The caterpillars were all dead in less than forty-eight hours. The number of inoculated potato beetles and their grubs which died did not exceed the number dead in the check injected with distilled water, so we must conclude that this beetle was not susceptible to the disease.

Apparently the activities of the insect and other animal parasites of the diseased locusts were not affected. We were able to rear several sarcophagid flies from diseased locusts and a very large number of living Gordioidea emerged from the diseased or dead insects.

Other workers have tested the pathogenicity of *Coccobacillus acridiorum* for various other insects and have found that not all insects are susceptible to the disease.

D'Herelle found that chickens, guinea pigs and rabbits were not susceptible and that man apparently suffered no ill effects even when the cultures were carelessly handled.

E. Experiments in the Laboratory.

Experiments were performed in the laboratory in order that we might become acquainted with the nature and action of the disease before trying it out in the field. These experiments were all carried out in breeding cages which were sterilized before each experiment.

The number of animal parasites, chiefly nematodes, and Diptera, was exceedingly high, so it must be borne in mind that several of the deaths recorded in these experiments may have been due entirely to the parasite or to the fact that the resistance of the locusts was lowered owing to the weakening action of the animal parasites.

Deaths which did not occur within a week to ten days were considered doubtful because the percentage of deaths among the checks confined for so long a time was fairly high.

Experiment 1. Effect of Spraying the Insect with a Culture of Coccobacillus.

Ten locusts were sprayed thoroughly. One died at the end of thirty hours, a second in two days, a third in three days, and at the end of eight days there were only five dead. The others remained alive for some time showing no symptoms of disease.

Experiment 2. Effect of Contaminating the Soil.

a. Twelve locusts were placed in a breeding cage containing sand sprayed with a culture of *C. acridiorum*. One died at the end of the first day. The others remained alive for several days and showed no symptoms of disease.

b. Several locusts were placed in an unsterilized cage from which dead locusts had just been removed. No mortality was produced.

Experiment 3. Effect of Contaminating the Food of the Locusts.

a. Seventeen nymphs were placed in a cage containing green food sprayed with a culture of the organism. The food was renewed daily and for several days it was sprayed either with a pure culture of the coccobacillus or with a suspension of the intestines of dead locusts. There were no deaths until the fifth day, when one nymph died. After this there were a few deaths at intervals. The experiment was discontinued at the end of three weeks. The intestines of some of the living locusts were then examined and *C. acridiorum* was found.

b. Twenty locusts were fed with sweetened bran mash to which a culture of the coccobacillus had been added. Two died during the next day. By the seventh there were altogether twelve dead and on the eleventh day fifteen. The others survived for eight days after being removed to a clean cage.

Experiment 4. Infection from Dead or Diseased Locusts.

Experiments were tried to determine whether the disease would spread readily from dead or diseased locusts to healthy ones. To this end a number of healthy locusts were placed in a cage with others that had just died. The locusts used were largely *M. femur-rubrum* with a few individuals of other species. Nearly all the locusts failed to show symptoms of the disease.

It was observed that occasionally a *bivittatus* would feed on the dead insects. In order to determine the effect of this cannibalistic tendency on the spread of infection we placed *femur-rubrum* and *bivittatus* in equal numbers in a cage with dead locusts. At the end of eight days 80% *bivittatus* were dead and only 20% *femur-rubrum*. We have never in our experiments observed any manifestation of cannibalism in any of the forms of locusts and grasshoppers experimented on except *M. bivittatus*, and in this case the tendency to prey on the feeble individuals is not very marked.

Experiment 5. Relative Resistance of Male and Female Locusts.

We were unable to observe any difference in the resistance of male and female locusts. The following is an example which shows how similar the two sexes are in the degree of susceptibility:

TABLE III.

Sex	No. inoculated	NUMBER DEAD IN					
		10 hrs.	12 hrs.	14 hrs.	15 hrs.	16 hrs.	20 hrs.
Female....	10	4	5	7	8	9	10
Male.....	10	5	6	7	8	9	10

Experiment 6. Relative Resistance of Adult and Nymph.

It was observed during the various experiments that the nymphs apparently were more resistant than the adults. Two experiments to definitely prove this gave the results shown in the following table:

TABLE IV.

Stage	No.	NUMBER DEAD IN							Alive at end of 13 hrs.
		3 hrs.	6 hrs.	8 hrs.	9 hrs.	11 hrs.	12 hrs.	13 hrs.	
Adult.....	10	1	3	6	9	..	..	..	1
Nymph.....	10	..	..	1	2	3	4	5	5

TABLE V.

Stage	No.	NUMBER DEAD IN							
		10 hrs.	11 hrs.	12 hrs.	13 hrs.	14 hrs.	16 hrs.	18 hrs.	23 hrs.
Adult.....	10	2	5	9	10	..	..	..	..
Nymph.....	10	2	..	3	..	4	6	7	9

Experiment 6. Relative Susceptibility of Different Species.

Experiments were tried to ascertain whether there was any difference in the susceptibility of *M. femur-rubrum*, *M. bivittatus*, *D. carolina* and *S. curtipennis*. In no two experiments did the results accord, so we concluded that, as far as these four species are concerned, differences in susceptibility are individual rather than specific.

Similar results were obtained when we tried to ascertain whether any one species was more susceptible to a culture obtained from the same species or from a different species.

From the results of the foregoing experiments it is clear that *Coccobacillus acridiorum* is pathogenic to all the common injurious locusts and grasshoppers of Eastern Canada, and that these insects are equally susceptible. The immature stages of the insects are more resistant than the adult stage.

Infection does not spread readily to healthy insects by mere contact with diseased locusts or other contaminated material. The chief, if not the only method of spreading the disease, is by ingestion of infected material.

While many individuals are tolerant of a mild infection they are not totally immune because all the locusts which survived in the various experiments succumbed when re-inoculated with a strong virulent culture. Their tolerance is probably due to the presence of certain closely allied bacilli in the intestines.

F. Experiments in the Field.

Experiments in an Enclosed Area:

In order to be definitely certain of our results a small area of a lawn was enclosed with screen-wire and numerous locusts included in the enclosure.

Experiment 1.—The grass of the enclosed area was sprayed with a bouillon culture of *C. acridiorum* and daily observations made for a week. During this time not a single death was recorded. The failure of this attempt was probably due to the death of the organism as a result of its exposure to bright sunlight. As it remained very bright for some time after this the experiment was not repeated.

Experiment 2.—A new portion of the lawn was enclosed and sown with sweetened bran mash to which a bouillon culture of the organism had been added. On the second day we found 21 dead locusts, and several others showing symptoms of the disease. At the end of five days we had collected altogether 108 dead locusts. Many of the survivors were then placed in insect cages and the majority died within five days of their capture.

Experiment 3.—Twenty locusts inoculated with a virulent culture of *C. acridiorum* were introduced among the healthy locusts in another enclosed area. At the end of the fifth day only 39 dead locusts, including the inoculated ones, were found. The experiment was continued for several days but no further deaths were recorded.

Experiments in the Open Field:

Two unsuccessful attempts were made to create an epizootic centre in the open field.

Experiment 1. The first attempt was made in a clover field badly infested with *M. femur-rubrum*. A small area of this field was treated with the infected bran mash. The field was examined daily but comparatively few dead locusts and no evidences of an epizootic were found. Numbers of locusts were collected from this field and placed in insect cages but the disease did not develop among them.

Experiment 2. A similar experiment was conducted on a badly infested lawn with the same results.

G. Conclusions.

The results of our work indicate that d'Herelle's biological method for the control of locusts cannot take the place of the methods now in use under the conditions which obtain in Eastern Canada. Should the disease become established, its spread would be extremely slow owing to the non-migratory and non-cannibalistic habits of the native species. The ideal conditions for the effective use of this method are those such as d'Herelle and others found in South America and North Africa where the locusts were in quickly moving swarms and were markedly cannibalistic in their habits. Indeed, most of these writers have emphasized the fact that "acridiophagy" is the chief factor in the spread of the disease. Another hindrance to the effective use of this method lies in the presence of several native strains of a coccobacillus identical with or closely related to d'Herelle's. These organisms are undoubtedly responsible for the immunity of the locusts to a mild infection of *Coccobacillus acridiorum*.

PART II. DESCRIPTIVE STUDIES ON COCCOBACILLUS ACRIDIORUM D'HERELLE, AND SIXTEEN RELATED NATIVE ORGANISMS.

During the early part of our work we made plates daily from the intestinal contents of dead locusts. In every case we got a pure culture of the organism. The culture medium used was 1% beef peptone agar and the plates were kept at room temperature (about 30° C.). The growth under these conditions is rapid. The colonies are spreading and filmy and not

as sharply defined and compact as they appear in a more concentrated agar. The typical colonies appeared within ten hours and the culture was always ready for use within 18 hours.

An attempt was made to estimate the number of viable organisms found in the digestive tract of insects which had died from the disease and also of those which survived infection. As one would expect, the number of coccobacilli in the intestines of dead locusts varied between very wide limits, depending probably on the length of time elapsing between infection and death, and on the number of organisms originally injected. The number usually exceeded 100,000 and our experiments showed that this number continued to increase after the death of the host.

4 hours after inoculation, just dead, 100,000 organisms.
10 hours after inoculation, 6 hours dead, 400,000 organisms.
24 hours after inoculation, 20 hrs. dead, 5,000,000 organisms.

Locusts which survived infection gave a much lower count, as the following table shows:

TABLE VI.

NUMBER OF COCCOBACILLI IN INTESTINAL TRACT OF LOCUSTS SURVIVING INFECTION.

SOURCE OF INFECTION	NUMBER OF COCCOBACILLI
Weak culture *Coccobacillus*	150
Stronger culture *Coccobacillus*	1,600
Infected food in laboratory	1,500
Contact with dead locusts	200
Infected food in enclosed field	30,000

Viability of Coccobacillus acridiorum in Bran Mash.

If bran mash is used for the conveyance of the coccobacillus it would be important to know how long the organism will retain its virulence in the mash. To test this we placed a shallow receptacle of bran in the shade out of doors. On the first day there were 365 million coccobacilli per gram of bran mash. After four days the number was reduced to 100 million, and a few of the locusts which were fed this mash died. At the end of eight days there were 250,000 coccobacilli per gram of bran mash. Locusts injected with a pure culture of coccobacilli from the eight day old bran did not die.

Native coccobacilli isolated from the digestive tract of Locusts.

In the first part of this paper we stated that our failure was probably due in part to the immunizing effect of native strains of coccobacilli.

We have described altogether sixteen organisms, some practically identical with d'Herelle's organism, the others more or less closely related.

The first culture was obtained from an individual of *Melanoplus bivittatus* which dropped dead near one of the authors at some distance from the laboratory, before any experiments were tried in the field. This coccobacillus showed a progressive increase in virulence similar to *Coccobacillus acridiorum*.

The other cultures we obtained both from apparently healthy locusts and from diseased or dead ones. In addition to those described a few other organisms were isolated, but we have included only those which are allied to *Coccobacillus acridiorum* and which injected into the intestinal canal of locusts cause death within twenty-four hours.

TABLE VII.

SOURCE, NUMBERS, ETC., OF THE COCCOBACILLI DESCRIBED.

Culture	Source	Location	Total No. of organisms	Coccobacilli	Other organisms
3	M. femur rubrum (healthy)	Montreal Island	4,000	4,000	.....
5	D. carolina (healthy)	"	7,000	7,000	.....
13 & 14	M.femur-rubrum "	"	1,400	1,400	.....
12	" " "	"	4,400	4,400	.....
15	" " "	"	3,500	3,000	500
6	M. bivittatus "	"	6,800	6,750	50
10	D. carolina "	Islet in Lake St. Louis	7,000	7,000	.....
11	S. curtipennis "	"	840	840	.....
8	M.femur-rubrum "	"	560	560	a few developed later
17	" " "	Mainland	4,400	4,400	.....
7	D. carolina (dead)	Montreal Island	600,000	600,000	.....
2	M. bivittatus (dead)	"	200,000	200,000	.....
4	" (diseased)	"	60,000	60,000	.....
16	" (parasitized by maggot)	"	200	60	180

We include below d'Herelle's original description as well as a fuller description by ourselves of the culture received from the Pasteur Institute. For convenience the organisms are divided into four groups. The first group includes *C. acridiorum* and those native coccobacilli which are practically identical with it; the second and third groups include strains which differ in several details, and the fourth group includes two organisms which differ chiefly in the fact that they are able to liquefy gelatine.

Coccobacillus Acridiorum d'Herelle.

Original Description by d'Herelle.

Morphology. Short bacillus, slightly oval, polymorphous. Cocci 0.6μ, bacilli 0.4μ–0.6μ by 0.9μ–1.5μ. Very motile. Flagella peritrichiate. Stains easily. Gram negative.
Agar Stroke. Not mentioned.
Potato. Growth abundant, creamy. Condensation water, syrupy and strongly alkaline.
Gelatine Stab. Not mentioned.
Nutrient Broth. Turbidity apparent after 4 hours, no sediment, clearing after 3 weeks with slight sediment, odor of beef extract.
Milk. Coagulated, strong alkaline reaction.
Litmus Milk. Not mentioned.
Gelatine colonies. No liquefaction.
Agar colonies. Circular in shape, waxy, visible after 12 hours, 18 hours, 3 mm. diameter. *Below surface* small, spherical, whitish opaque.
Aesculin agar. Not mentioned.
Fermentation of Sugars. +glucose, levulose, galactose, maltose. No other sugars mentioned.
Indol. Not mentioned.
Neutral red bilesalt Agar. Not mentioned.
Pathogenicity. Pathogenic to various Acrididæ, ants and caterpillars.

GROUP I.

Culture of C. acridiorum from Pasteur Institute, and Cultures 6, 7, 13 and 14.

Morphology. From agar slope 20 hours old, short rods or cocci, some oval, polymorphous. 0.7μ–1.0μ. In milk culture they appear often as diplococci. Motile. Gram—. Amylgram+. Stain readily.
Agar stroke. Abundant growth, spreading, flat, glistening, smooth, dirty white to bluish white, opaque, butyrous, medium unchanged. On 1% agar the cultures are arborescent and transparent.
Potato. Abundant growth, spreading, flat, glistening, smooth, butyrous; color from dirty white to yellow.
Gelatine Stab. Uniform growth, line of puncture filiform. No liquefaction, medium unchanged. Stab brownish yellow.
Nutrient Broth. Pellicle or ring, turbidity, slight sediment, no clearing after 14 days, odor of beef extract.
Milk. At first gas production without coagulation. Delayed coagulation in 2–8 days, acid reaction after 8 days, no peptonisation, medium unchanged, no extrusion of whey.
Litmus Milk. Gas production, weak acidity, no reduction. After 4 days partial to complete coagulation, acid.
Gelatine Colonies. Growth slow, round, raised, edge entire, yellow. 3 weeks, 2 mm. diameter, yellow white. No liquefaction.

Agar colonies. Rapid growth, irregular, round, smooth, flat, edge entire, amorphous, dirty white to blue transparent. Growth more restricted on 1½% than on 1% agar.

Aesculin bilesalt agar. C. acridiorum weak field after 24 to 48 hrs. Cultures 6, 7 and 13 typical black field after 24 hrs., greatly increased in intensity after 48 hrs. Culture, 14, no field after 24 hrs., very intense black field after 48 hrs.

Fermentation of sugars. +glucose, galactose, muscle sugar, lactose (weak). —adonit, dulcit.

Differences are observed in the following sugars:

	C. Acrid.	Cult.6	7	13	14
Saccharose	+	+	+	+	—
Rafinose	+	+	+	+	—
Arabinose	+	—	—	+	+

Indol reaction. Negative.

Neutral red bilesalt agar. C. acridiorum strong fluorescence, red, spreading.
Culture 6, strong fluorescence, canary yellow, spreading.
Culture 7, strong fluorescence, canary yellow.
Culture 13, strong fluorescence, canary yellow, red ring, spreading.
Culture 14, strong fluorescence, canary yellow, red ring, spreading.

Pathogenicity. Pathogenic to locusts and grasshoppers. Injection fatal within 24 hours.

GROUP II.

Cultures 4, 10, 12, 20.

Morphology. From agar slope 20 hrs. old. Short rods or cocci, polymorphous. 0.7μ–1.0μ. In milk culture they appear as micrococci or diplococci. Decidedly motile. Gram—. Amylgram+, except Culture 12, which is negative. Stain easily.

Agar stroke. Abundant growth, spreading, flat, glistening, smooth, dirty white to bluish white, opaque, butyrous, medium unchanged. On 1% agar the cultures are arborescent and transparent.

Potato. Growth abundant, spreading, flat, glistening, smooth, butyrous, color from dirty white to deep yellow. Culture 12 has a drier growth than the other cultures.

Gelatine Stab. Growth uniform, line of puncture filiform, of a yellow brown color, no liquefaction, medium unchanged. Culture 20 started to branch after 14 days, the stab was white in color.

Nutrient broth. Culture 4, Pellicle, turbidity, sediment, no clearing after 14 days.
Culture 10, Ring, turbidity, sediment. Clearing after 48 hrs., odor of beef extract.
Culture 12, Ring, turbidity, no sediment, no clearing, after 14 days but slight sediment.
Culture 20, Turbidity, sediment, no clearing.

Milk. Gas production without coagulation. Delayed coagulation in 3 days, except in case of Culture 10, which did not coagulate at all, acid reaction, no peptonisation, medium unchanged, no extrusion of whey.

Litmus Milk. Gas production with weak acidity, no reduction. After 4 days acid and complete coagulation except Culture 10, which remained neutral to slightly alkaline and liquid.

Gelatine colonies. Culture 4, growth moderate, brownish white, round, convex, entire, no liquefaction, size 1 mm.
Culture 10, heavy growth, bright yellow, round, convex, entire, no liquefaction, 1 mm.
Culture 12, very slow growth, white punctiform, no liquefaction.
Culture 20, slow growth, bluish white, punctiform, no liquefaction.

Agar colonies. Rapid growth, round, flat, edge entire, internal structure amorphous, blue transparent.

Aesculin agar. Culture 4, decided black field after 24 hours; very strong after 48 hours.
Culture 10, no growth 24 hours, slight growth, weak field 48 hours.
Culture 12, decided field after 24 hours, very strong after 48 hours.
Culture 20, no field after 48 hours, good growth.

Fermentation of sugars. —dulcit, raffinose.
+saccharose, glucose, lactose, muscle sugar, galactose.
Culture 10 in general causes very little fermentation, only traces in saccharose and glucose.
Differences are shown—

	4	10	12	20
Adonit	+	—	+	—
Arabinose	+	—	—	+

Indol reaction. Negative.
Neutral red bilesalt agar. Culture 4, fluorescence, canary yellow, slightly spreading.
Culture 10, slight fluorescence, canary yellow.
Culture 12, strong fluorescence, canary yellow.
Culture 20, canary yellow, red ring.
Pathogenicity. Pathogenic to locusts. Death by injection occurs within 24 hours.

GROUP III.

Cultures 2, 3, 5, 15, 16, 17.

Morphology. From agar slope 20 hrs. old. Short rods or cocci, polymorphous. 1.5μ–0.9μ. In Milk culture they appear as small or large micrococci. Very motile. Gram—. Amyl gram, cultures 2, 5, 15, 17 negative, cultures 3, 16+. Stain easily.
Agar stroke. Abundant growth, spreading, flat, glistening, smooth, dirty white, opaque, butyrous, medium unchanged. On 1% agar cultures are aborescent and transparent.
Potato. Cultures 2 and 17 growth abundant, spreading, flat, glistening, smooth, dry and brittle, yellow color. Cultures 3, 5, 15 and 16 abundant growth, spreading, flat, glistening, smooth, butyrous, dirty white to yellow.
Gelatine stab. Growth uniform, line of puncture filiform, of a yellow brown color, no liquefaction, medium unchanged.
Nutrient Broth. Pellicle or ring, turbidity, no clearing after 14 days, slight sediment, strong odor of beef extract.
Milk. Gas production without coagulation. Delayed coagulation except Culture 17, liquid and acid after 8 days.
Litmus Milk. Gas production with weak acidity. Cultures 5, 16 and 17 complete reduction; Cultures 2, 3, 15 no reduction. Cultures 2, 5, 16, 17 coagulation within 4 days; Cultures 3 and 15 no coagulation, acidity in Culture 3, neutral reaction in Culture 15.
Gelatine Colonies. Cultures 2, 3 and 5 slight growth, punctiform, white, no liquefaction. Cultures 15, 16 and 17 good growth, size 2 mm., bright yellow, round, convex, entire, no liquefaction.
Agar colonies. Rapid growth, round, flat, edge entire, internal structure amorphous, blue transparent, below surface small, spherical, whitish, opaque.
Aesculin agar. Culture 2 negative, Culture 15 no growth, Cultures 3, 16 and 17 positive, Culture 5 very weak.
Fermentation of sugars. +galactose.

	Adonit	Dulcit	Raffinose	Arabinose	Muscle sugar	Saccharose	Lactose	Glucose
Culture 2	—	+	—	+	+	+	+	+
Culture 3	+	+	+	+	+	—	+	?
Culture 5	—	—	—	+	—	+	+	+
Culture 15	—	—	—	—	—	+	—	?
Culture 16	—	—	?	+	+	+	+	+
Culture 17	—	?	—	+?	+	+	+	?+

Indol reaction. Cultures 2 and 3 very strong. Cultures 5, 15, 16, 17 negative.
Neutral red bilesalt agar. Cultures 2 and 3, red.
Culture 5, fluorescence, canary yellow, spreading.
Cultures 15 and 17, fluorescence, canary yellow.
Culture 16, canary yellow, red ring.
Pathogenicity. Pathogenic to locusts and grasshoppers. Death by injection within 24 hours.

GROUP IV.

Cultures 8 and 11.

This group shows much resemblance to Group III, but its ability to liquefy gelatine made it necessary to separate the two groups.

Morphology. From agar slope 20 hrs. old, short bacillus, slightly oval, polymorphous, 0.5μ–1.0μ. Very motile. Gram—. Amyl gram+. Stain easily.

Agar stroke. Abundant growth, spreading, flat, glistening, smooth, dirty white, opaque, butyrous, medium unchanged. Arborescent on 1% agar.

Potato. Growth abundant, spreading, flat, dull, smooth, butyrous, white.

Gelatine Stab. Growth rapid, liquefaction along puncture, on top saucer shape after 24 hours, completely liquefied in 7 days.

Nutrient broth. Culture 8 pellicle, turbidity, sediment, strong odor of beef extract. Culture 11, ring, turbidity, strong odor of beef extract. No clearing after 14 days.

Milk. Coagulation prompt, strong acid and gas, no extrusion of whey, medium unchanged.

Litmus Milk. Acid coagulation, no reduction, slow peptonisation.

Gelatine colonies. Complete liquefaction within 24 hours.

Agar colonies. Rapid growth, round, smooth, flat, edge entire, amorphous.

Aesculin agar. Negative.

Fermentation of Sugars. Negative. —Adonit, dulcite, galactose, arabinose, muscle sugar, lactose, raffinose. Weak, saccharose, glucose.

Indol reaction. Weak.

Neutral red bilesalt agar. Strong fluorescence, canary yellow.

Pathogenicity. Pathogenic to locusts. Death occurs within 24 hours of injection.

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